REMARKS

Status of the Application and Claims

With entry of this amendment, claims 1-9, 11 and 13-15 have been amended. Claims 1-15 are pending in the application. No new matter has been added by way of these amendments.

I. Claim Rejections - 35 U.S.C. § 112, second paragraph

The claims have been rejected for failing to particularly point out and distinctly claim the subject matter which the applicant regards as his invention. (Office Action, p. 2). The Applicants' amended their claims to better clarify their invention, incorporating wherever necessary the examiner's suggested claim language. *Id.* at pp. 2-3. Accordingly, each of the claims fully comply with the requirements of 35 U.S.C. § 112, second paragraph.

The phrase "modified," as used in claims 13 and 15, is to be given its ordinary meaning by one skilled in the art. For example, a modified alliinase gene can be a gene that over-expresses, temporally or spatially expresses, or has suppressed expression of its gene product.

Finally, the multiple claim dependencies in claims 4-5 and 8-16 have been amended.

II. Claim Rejections - 36 U.S.C. § 112, first paragraph

The examiner rejects dependent claims 13-15 under 35 U.S.C. § 112, first paragraph as not enabled for a plant transformed with a modified alliinase gene or a plant transformed with a modified gene in the sulfur pathway. (Office Action, p. 4.) In

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light of the examiner's admission that the invention is enabled for "transformed onion plants by the method of delivering DNA by vector or direct gene transfer, selecting transformed plant material and culturing and regenerating transformed onion plants" (*id.*), which is of greater breadth of scope than claims 13-15, the applicants respectfully traverse.

"A lack of enablement rejection under section 112, ¶ 1 is appropriate where the written description fails to teach those in the art to make and use the invention **as broadly as it is claimed** without undue experimentation." *In re Cortright*, 165 F.3d 1353, 1356, 49 USPQ2d 1464, 1466 (Fed. Cir. 1999) (emphasis added). The broadest claims on this applicant's invention are claims 1-12. These claims are admitted as enabled. (Office Action, p. 4.) If, as the examiner states, that "the state of the art is such that the skilled person can introduce a gene into plant cells, and given the appropriate regulatory signals and substrates, have a reasonable expectation of expressing the gene in transgenic plant tissue" (*id.* at p. 5-6) and the applicant teaches those in the art to make transformed onion plants (Office Action, p. 4), then the applicant has met its burden under 35 U.S.C. § 112, first paragraph without more. *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 U.S.P.Q. (BNA) 81, 94 (Fed. Cir. 1986) (enablement is "not precluded even if some experimentation is necessary, although the amount of experimentation needed must not be unduly excessive.")

Following the teachings of the specification with respect to, for instance, the transformation procedure as outlined in claims 1-12, it would be relatively easy for a person skilled in the art of molecular biology and transformation to put claims 13 to 16

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into practice using routine techniques well known in the art and without undue experimentation. *See Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 1532 (Fed. Cir. 1987) (holding that the enablement requirement looks to the objective knowledge of one of ordinary skill in the art).

To demonstrate that the scope of claims 1-12 is sufficient for a person skilled in the art of transformation to make transgenic alliums, the applicants represent that the technique is now being used by two commercial companies in the United States to transform different species of allium. At least one of those companies uses the technique from the description in this patent application. Additionally, outside the United States, the applicants represent that a researcher in Slovenia employed the teachings of the application and currently transforms onion with a modified gene from the onion carbohydrate pathway in order to modify plant phenotype in exactly the ways claimed in 13, 14, and 15. Each demonstrates further that the claims are enabled.

Additionally, once the scope of the invention is enabled, there is no need to "provide guidance on how to predictably eliminate inoperable embodiments . . . ." *In re Vaeck*, 947 F.2d 488, 496 (Fed. Cir. 1991) (stating that "[i]t is well settled that patent applicants are not required to disclose every species encompassed by their claims, even in an unpredictable art.") Nevertheless, in addition to the examiner's statement that a skilled person could introduce a gene into a plant to achieve a transgenic plant (Office Action, p. 5), standard techniques for cloning and isolating particular genes or gene sequences, are available publicly from, for instance, the NCBI databases[1]. Also,

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[1] *See* www.ncbi.nim.nih.gov.

many cassette systems are available into which genes or sequences can be cloned in order to effect increased, temporal, and spatial expression (e.g. pBin 19), or silencing of expression[2]. Binary vectors, containing selectable genes and reporter genes, are available (e.g. the pCAMBIA series from CSIRO, Australia, or pBIN series from Clontech) into which these 'cassettes' can be easily cloned and then used to transform Allium plants. Further, techniques are and have been readily available[3] that are effective in ~90% of transformations for specific silencing of genes.

The use of the above technologies would ensure that in the majority of transformations with modifications would behave as expected. Thus inoperable embodiments of the system would actually be quite rare. In the applicant's experience they have seen two definite unpredicted results from ~60 separate transgenic events[4]. Events that have been studied in onion have been predictably inherited as expected. Confirming that the transgene is being transcribed (producing mRNA) and translated (producing protein) as expected is also a straightforward procedure. Transcription levels are readily detected and quantified by use of RNA isolation Kits (e.g. RNAeasy

[2] E.g. Wesley, S.V., C.A. Helliwell, et al, (2001). "Construct design for efficient, effective and highthroughput gene silencing in plants," The plant journal 27(4): 1-12).

[3] (Smith, N.A., S.P. Singh, et al. (2000). "Total silencing by intron-spliced hairpin RNAs." Nature **407**: 319-320) and in widespread use (Hannon G.J. (2002). RNA interference [review]. Nature. 418(6894):244-251).

[4] (C.C. Eady, S. Davis, J. Farrant, J. Reader and F. Kenel (2003b). *Agrobacterium tumefeciens*-mediated transformation and regeneration of herbicide resistant onion (Allium cepa) plants. Annals of Applied Biology vol. 142 (in press)). (Eady C.C. (2002). Genetic transformation of onions in: Allium Crop Science: recent advances. Editors Rabinowitch H.D. and Currah L. CABI Publishing ISBN 0.851995101). (Eady C.C. (2002). The transformation of onions and related alliums in: Transgenic plants and crops. Editors Khachatourions et al., Publisher Marcel Dekker ISBN 0-8247.0545.0)

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from Qiagen) and RT-PCR kits to assay relative expression of a particular gene against a known gene, e.g. Ready-to-go™ RT-PCR beads from Amersham Bioscience http://www.amershambiosciences.com). Translation kits are and were likewise readily available (e.g. Invitrogen 'Westernbreeze' kit). The applicant has shown that each works effectively on transgenes in onions[5].

The applicant respectfully requests the objection be removed.

III. Claim Rejections - 35 U.S.C. §§ 102 and 103

The examiner rejects claims 1-4, 6, and 8-12 under 35 U.S.C. § 102 as anticipated by Bidney (EP0486234, published 20 May 1992), and under 35 U.S.C. § 103 as obvious by Bidney in view of the prior art. The applicant respectfully traverses.

The examiner has raised the above citation as an argument that the claims in this application are not novel because EP0486234 teaches "a method of transforming Allium plants comprising delivering previously manipulated DNA into embryos via a binary vector, selecting transformed material, and culturing and regenerating transformed plants. (Office Action, p. 7) (internal citations omitted.) Further, the examiner states that Bidney teaches "the method of using Agrobacterium, onions, the use of immature embryos, Agrobacterium having a selectable marker, herbicide resistance gene, bar resistance, an antibiotic resistance gene and a nptII gene." (*Id.*) (internal citations omitted.)

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[5] (C.C. Eady, J. Reader, S. Davis and T. Dale (2003a). Inheritance and expression of introduced DNA in transgenic onion plants (*Allium* cepa). Annals of Applied Biology vol. 142 (in press)

Anticipation

In order to properly anticipate claims 1-4, 6 and 8-12 under § 102, Bidney taken individually, must explicitly disclose each and every limitation recited in the claims. *See* M.P.E.P. § 2131. If Bidney, however, fails to expressly set forth a particular limitation, the examiner must show that this limitation is inherently disclosed to substantiate a claim of anticipation. See In re Robertson, 169 F.3d 743, 745 (Fed. Cir. 1999).

The teachings of Bidney offer only a general introduction to transformation techniques in plants, much like the initial publication by Horsch, R., J. Fry, et al, (1985), entitled "A Simple and General Method For Transferring Genes Into Plants[6]" whereby the concept of using Agrobacterium as a vector to deliver DNA and antibiotics (selectable agents) to detect and regenerate transgenic plants was created. Bidney implies that his method can be used with approximately 75 other plant species. Even though Bidney indeed lists onion (not other allium) in its compendium of plants, it does not provide any transformation details. For instance, Bidney does not specify which onion tissue should be used for transformation. In fact, there are up to 12 different tissue sources indicated in total with no direction or indication as to which would be suitable for onion.

The examiner suggests that Bidney teaches the use of embryos as provided by the applicants' invention. (Office Action, pp. 7-8.) The applicant uses a process, however, that is wholly different from that implicated by Bidney. Bidney discloses a transformation techniqu - were callus is first generated from embryos and then

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[6] Science 227: 1229-1231.

transformed. The applicants' process is distinct from this as they directly co-cultivate embryo tissue and then directly regenerate embryogenic cultures from this without a callus phase. One skilled in the art would not have considered such a concurrent transformation technique. Applicants assert that only subsequent to the applicants invention has a direct technique of agro-transforming and regenerating embryos from differentiated cultures been employed to replace standard culturing . In fact, even today this move towards directly transforming tissues with a minimum of tissue culture[7] is only routinely used for the model plant species *Arabidopsis*, perhaps the most studied plant known.

Bidney also teaches the delivery of *Agrobacterium* via the use of microparticles. The applicant has carried out sufficient research to demonstrate that this (microparticle) method of wounding plant cells will not deliver the vector or DNA to cells that are both competent to receive and integrate foreign DNA and subsequently be capable of regenerating into a complete fertile plant. Research in the applicant's laboratory from 1994 to 1996 focused on the development of a microprojectile based transformation system for Alliums. Using state of the art Bio-rad particle gun and a reporter gene construct that was highly expressed in allium tissue they effected hundreds of bombardments into over ten thousand embryos or embryo derived cultures. This delivery system delivered DNA 17 to 34 fold more effectively then *Agrobacterium* did to

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[7] E.g. G. Hanson and M.S. Wright (1999), Recent advances in the transformation of plants. Trends in Plant Science, Vol. 4, No. 6, pp. 226-231.

the same tissue[8]. On no occasion did this delivery system result in the successful transformation and regeneration of a complete onion plant. The reason for this is due mainly to biolistics, not being able to target the correct progenitor cells that are capable of accepting DNA and regenerating. The applicant's system of isolating embryos at a specific stage of development, on the other hand, and either directly wounding or culturing and wounding these cell types is a novel development, which, when combined with their particular Agrobacterium co-cultivation procedure, results in the delivery to correct tissues.

Once again, the applicants assert that only subsequent to their application have groups developed cell lines in Allium (that are both competent to receive DNA and regenerate and, therefore, more like the embryo cultures specified in our application). These subsequent cultures are different to those recommended by Bidney in that they are not direct explants (as preferred by Bidney - EP0486234, page 4, line 12) or callus. Rather, they are cell cultures (similar to our claim) of either meristem by *Agrobacterium*-mediated gene transfer[9] or embryo origin[10]. Neither of these methods use Agrolistic techniques to effect transformation.

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[8] (Eady, C.C., C.E. Lister, et al. (1996), "Transient Expression of *Uida* Constructs in *in Vitro* Onion (*Allium Cepa* L) Cultures Following Particle Bombardment and *Agrobacterium*-Mediated DNA Delivery." Plant Cell Reports, 15(12): 958-962).

[9] Kondo, T., H. Hasegawa, et al. (2000). *Genetic Transformation and Hybridization*: Transformation and regeneration of garlic (*Allium sativum* L.), Plant Cell Rep. 19(10): 989-993.

[10] Zheng SJ., Khrustaleva L., Henken B., Sofiari E., Jacobsen E., Kik C., Krens FA. (2001). Agrobacterium tumefaciens-mediated transformation of Allium cepa L.: the production of transgenic onions and shallots, [Article] *Molecular Breeding*. 7(2): 101-115.

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Furthermore, as Bidney is a general reference, there is no indication about which plant media should be used for embryogenic culture induction and regeneration. Nor is there mention of which selection agent or levels should be used to discriminate between transgenic and non-transgenic tissue for any species, let alone onion. There is no indication of time on selection is given or when to regenerate back to plants.

Obviousness

The examiner also rejects claims 1-6 and 8-12 under 35 U.S.C. § 103 as being obvious over Bidney. (Office Action, p. 8.) To do so, the examiner must establish a prima facie case of obviousness by demonstrating that (1) Bidney discloses or suggests each and every limitation recited in the above claims, (2) there is reasonable probability of success of any modification of the teachings of Bidney, and (3) the existence of some suggestion or motivation, either in the teachings of Bidney itself or in the knowledge generally available to one of ordinary skill in the art, to make such a modification in a manner resulting in the claimed invention. See M.P.E.P. § 2143.

For the same reasons stated above, Bidney cannot disclose the applicants' invention.

For these reasons, the present claims are neither anticipated nor made obvious by Bidney and the applicant respectfully requests that the rejections be removed.

In view of the foregoing amendments and remarks, Applicant respectfully requests the reconsideration and reexamination of this application and the timely allowance of the pending claims.

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Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

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